08/403,844

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Claims:

Please amend claims 22 and 87 as follows:

22. (Five Times Amended) A method for detecting a specific living target cell in a cell suspension of a mixed cell population, in a fluid system containing a mixed cell population, or in a cell suspension prepared from a solid tissue[; [redacted], with the exception of normal and malignant hematopoietic cells in blood and bone marrow, the method comprising the steps of:

a. coating paramagnetic particles or beads with a first antibody or antibody fragment directed against a second antibody or antibody fragment;

b. incubating the second antibody or antibody fragment with the cell suspension to bind the second antibody or antibody fragment with the target cell, thereby creating a cell mixture, wherein the second antibody or antibody fragment is directed against a membrane structure specifically expressed on the target cell and not on a non-target cell in the cell mixture;

c. washing the cell mixture to remove unbound second antibody or antibody fragment;

d. mixing the coated paramagnetic particles or beads with the washed cell mixture;

e. incubating the washed cell mixture and the coated paramagnetic particles under gentle rotation at about 4°C until target cell-bead rosettes are formed; and

g. visually detecting the target cell-bead rosettes after incubation;

wherein the target cells are living and can be detected at a sensitivity of one target cell per 100 or more total cells.

23. The method of claim ~~22~~ 1, wherein the paramagnetic particle or bead is coated with a monoclonal murine or a human antibody or fragment thereof.

24. The method of claim ~~22~~ 1, wherein incubating lasts for 5-10 minutes to 2 hours.

4 ~~25.~~ The method of claim ~~24~~ 3, wherein incubating lasts 30 minutes.

5 ~~28.~~ The method of claim ~~22~~ 1, wherein the method further comprises the step of:

pre-incubating the antibody-coated paramagnetic particle and the cell suspension with mild detergent.

6 ~~29.~~ The method of claim ~~28~~ 5, wherein the preincubating comprises as detergent polyoxyethylenesorbitan monolaurate at a concentration less than 0.1% and the preincubation lasts 30 minutes at 4°C.

7 ~~33.~~ The method of claim ~~22~~ 1, further comprising the steps of:

isolating the target cell-bead rosettes by applying a magnetic field to separate the rosettes.

8 ~~34.~~ The method of claim ~~22~~ 1, wherein the second antibody or fragment thereof is directed against an antigen or a receptor in a cell with abnormal developmental patterns.

9 ~~35.~~ The method of claim ~~34~~ 8, wherein the cell is a primary or a metastatic cancer cell.

11 ~~36.~~ The method of claim ~~22~~ 1, wherein the monoclonal antibody or fragment is of IgG isotype, a $F(ab')_2$ fragment, a F(ab) fragment, IgM, or a fragment of IgM.

12 ~~37.~~ The method of claim ~~22~~ 1, wherein the mixed cell population comprises mammalian tissue, a pleural effusion, a peritoneal effusion, a body fluid, or a solid tumor in a normal tissue or organ.

13 ~~38.~~ The method of claim ~~37~~ 12, wherein the mammalian tissue comprises human bone marrow or human peripheral blood; the body fluid comprises urine, cerebrospinal fluid, semen, or lymph; or the normal tissue or organ comprises liver, lymph node, spleen, lung, pancreas, bone, central nervous system, prostate gland, skin, or mucous membranes.

14 ~~39.~~ (Twice Amended) The method of claim ~~22~~ 1, wherein the second antibody or antibody fragment is directed against fibronectin receptor, β-integrin, vitronectin receptor, αγβ3-integrin, P-seletin including GMP-140, CD44-variants, N-CAM including CD-56, E-cadherin, Le^y, carcinoembryonic antigen or CEA, EGF receptor, c-erbB-2 including HER2, transferin receptor, TNF-receptor, high molecular weight antigen, p95-100, sarcoma antigens including TP-1 and TP-3 epitope, Mv 200kD, Mv160kD, MOC-31 epitope including cluster 2 epithelial antigen, MUC-1 antigen including DF3-epitope and gp290kD, prostate high molecular antigen, TAG 72, bladder carcinoma antigen, Mv 48kD colorectal carcinoma antigen, lung carcinoma antigen Mv 350-420kD, Mel-14 epitope. $ß_2$-microglobulin, Apo-1 epitope, or pan-human cell antigen.

15 ~~40.~~ The method of claim ~~22~~ 1, wherein the second antibody or antibody fragment is directed against a growth factor receptor or an oncogene product expressed on the membrane of a malignant cell.

10 ~~43.~~ The method of claim ~~34~~ 8, wherein the second antibody or antibody fragment is directed against breast, ovarian or lung carcinoma cells; melanoma, sarcoma, glioblastoma or cancer cells of the gastrointestinal tract; melanoma, sarcoma, glioblastoma or cancer cells of the genitourinary tract; or melanoma, sarcoma, glioblastoma or cancer cells of the reticuloendothelial system.

24 ~~46.~~ (Five Times Amended) A kit for performing the method of claim 22, the kit comprising:

a. a first antibody, wherein said first antibody is a specific monoclonal antibody or antibody fragment directed against a second antibody or antibody fragment, said first antibody coating a paramagnetic particle or bead without removing its antigen-binding ability;

b. a paramagnetic particle or bead; and

[redacted]

c. the second antibody, wherein said second antibody is a specific monoclonal antibody or antibody fragment directed against an antigen or a receptor within or on the target cell;

wherein said second antibody or antibody fragment is conjugated to a detectable label.

25 ~~57.~~ 24 The kit of claim ~~46~~, wherein the detectable label is an enzyme peroxidase or alkaline phosphatase.

16 ~~59.~~ (Twice Amended) The method of claim ~~22~~ 1, wherein the second antibody or antibody fragment directed against a membrane structure specifically expressed on the target-cell is a murine or a human antibody or fragment thereof.

17 ~~62.~~ (Four Times Amended) The method of claim ~~22~~ 1, wherein the method further comprises after incubating, applying a magnetic field to separate out the target cell-bead rosettes.

18 ~~64.~~ (Twice Amended) The method of claim ~~22~~ 1, wherein visually detecting includes counting the target cell-bead rosettes using a microscope or a cell or particle counting device.

28 ~~87.~~ (Four Times Amended) A method for detecting living tumor cells in a cell suspension of mixed cell population or in a cell suspension prepared from a solid tissue[, [redacted]], with the exception of normal and malignant hematopoietic cells in blood and bone marrow, comprising:

a) coating paramagnetic particles with a first antibody or fragment directed against a second tumor-specific monoclonal antibody or fragment;

b) incubating the second tumor specific antibody or antibody fragment with the cell suspension to allow the second tumor specific antibody or antibody fragment to bind the tumor cells;

c) washing the cell suspension to remove unbound second antibody or antibody fragment;

d) mixing the coated paramagnetic particles with the cell suspension;

e) incubating the mixture at about 4°C under gentle rotation until tumor cell-bead rosettes are formed; and

f) visually detecting the tumor cell-bead rosettes;

wherein the target cells are living and can be detected at a sensitivity of one target cell per 100 or more total cells.

29 ~~88.~~ (Amended) The method according to claim ~~87~~ 28 further comprising after incubating; applying a magnetic field to the mixture to separate out the tumor cell-bead rosettes.

30 ~~89.~~ (Amended) The method according to claim ~~87~~ 28, wherein the tumor-specific monoclonal antibody is specific for tumor antigens comprising a growth factor receptor, an oncogene product expressed on the membrane of a malignant cell, an adhesion membrane molecule, an MDR protein, breast, ovarian or lung carcinoma cells; melanoma, sarcoma, glioblastoma or cancer cells of the gastrointestinal tract; melanoma, sarcoma, glioblastoma or cancer cells of the genitourinary tract; or melanoma, sarcoma, glioblastoma or cancer cells of the reticuloendothelial system.

31 ~~96.~~ (Amended) The method according to claim ~~87~~ 28, wherein the mixture is incubated for about 30 minutes.

19 ~~101.~~ (Amended) The method according to claim ~~22~~ 1, further comprising after incubating; applying a magnetic field to the mixture to separate out the target cell-bead rosettes; and detecting target cell specific genes.

26 ~~106.~~ The kit of claim ~~46~~ 24, comprising a paramagnetic particle or bead coated with the first antibody and a paramagnetic particle or bead not coated with antibody.

20 ~~108.~~ The method according to claim ~~22~~ 1, wherein the first antibody or antibody fragment is a monoclonal antibody or antibody fragment, the second antibody or antibody fragment is a monoclonal antibody or antibody fragment, or the first and second antibodies or antibody fragments are monoclonal antibodies or antibody fragments.

21 ~~109.~~ The method according to claim ~~22~~ 1, wherein the visually detecting includes conjugating a detectable label to the second antibody.

22 ~~110.~~ The method according to claim ~~22~~ 1, wherein the target cells are detected at a sensitivity of one target cell per 1000 or more total cells.

23 ~~111.~~ The method according to claim ~~22~~ 1, wherein the second antibody is an IgG antibody and the first antibody recognizes the Fc-portion of the second antibody.

32 ~~116.~~ The method according to claim ~~87~~ 28, wherein the target cells are detected at a sensitivity of one target cell per 1000 or more total cells.

27 ~~117.~~ (Twice Amended) A kit for performing the method of claim 22, the kit comprising:

a. a first antibody, wherein said first antibody is a specific monoclonal antibody or antibody fragment directed against a second antibody or antibody fragment, said first antibody coating a paramagnetic particle or bead without removing its antigen-binding ability;

b. a paramagnetic particle or bead; and

c. the second antibody, wherein said second antibody is a specific monoclonal antibody or antibody fragment directed against an antigen or a receptor within or on the target cell, wherein the second antibody or antibody fragment is directed against fibronectin receptor, β-integrin, vitronectin receptor, αvβ3-integrin, P-seletin including GMP-140, CD44-variants, N-CAM including CD-56, E-cadherin, Le^y, carcinoembryonic antigen or CEA, EGF receptor, c-erbB-2 including HER2, transferin receptor, TNF-receptor, high molecular weight antigen, p95-100, sarcoma antigens including TP-1 and TP-3 epitope, Mv 200kD, Mv160kD, MOC-31 epitope including cluster 2 epithelial antigen, MUC-1 antigen including DF3-epitope and gp290kD, prostate high molecular antigen, TAG 72, bladder carcinoma antigen, Mv 48kD colorectal carcinoma antigen, lung carcinoma antigen Mv 350-420kD, Mel-14 epitope, $ß_2$-microglobulin, Apo-1 epitope, or pan-human cell antigen;

wherein said second antibody or antibody fragment is conjugated to a detectable label.

wherein said second antibody or antibody fragment is conjugated to a detectable label.

Claims ~~41, 42, 80-86, 90, 91, 94, 95, 97, 98, 100, and 104~~ are withdrawn from consideration.

41. The method of claim 40, wherein the monoclonal antibody or antibody fragment is directed against an insulin receptor, an insulin-like receptor, or IGF.

42. The method of claim 34, wherein the monoclonal antibody or antibody fragment is directed against an adhesion membrane molecule or an MDR protein in the abnormal cell.

80. (Amended) The method according to claim 22 further comprising after incubating; detecting a second antigen of the target cell by adding a second labeled monoclonal antibody directed to the second antigen to the cell suspension; and quantitating the amount of labeled second monoclonal antibody bound to the rosettes.

81. The method according to claim 80, wherein the second monoclonal antibody is specific for a tumor prognostic marker.

82. The method according to claim 80, wherein the second monoclonal antibody is labeled with fluoresceine, a radioactive compound, biotin, or an enzyme.

83. (Amended) The method according to claim 22 further comprising before mixing; prelabeling the target cells with a labeled second monoclonal antibody to second antigen on the target cell; and after incubating, quantitating the amount labeled second monoclonal antibody bound to the rosettes.

84. (Amended) The method according to claim 22, further comprising after incubating, applying a magnetic field to separate out the target cell bead rosettes; and detecting target cells specific genes at the DNA, mRNA or protein level.